Paper No. 14

TITLE: A RAPID GC-2 DETERMINATION OF HARMAN AND NORHARMAN IN SMOKE

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ABSTRACT: A method has been developed for the rapid analysis of the important bases, harman and norharman, in small quantities of cigarette smoke or pyrolyzates. Compared to previously reported procedures, the method greatly reduces the number of steps needed to isolate and quantitate these compounds, as it only requires glass capillary gas chromatography (GC-2) of a basic fraction of cigarette smoke condensate (CSC). Two variations of the method are possible depending upon the mode of collection of CSC. Method A involves collection of the smoke in dry ice-cooled traps and dissolving CSC in 30% MeOH/$CHCl_3$. Method B involves collection of smoke on Cambridge filter pads, with subsequent extraction of the TPM with an HCl/MeOH/$CHCl_3$ solution. CSC solutions (from either procedure) were then extracted three times with 2N HCl. The aqueous phase, containing bases, was adjusted to pH 12, saturated with salt, and extracted with $CHCl_3$ to give a "free base" fraction. Harman and norharman could be quantitated by direct GC-2 analysis of the base fraction on a WCOT fused silica column, coated with Superox-4. When added to CSC, harman and norharman were quantitatively recovered. The merits of the two methods of smoke collection will be discussed. The method was applied to the analyses of the smoke from different cigarettes and tobacco pyrolyzates. The levels of these two compounds in cigarette smoke appeared to follow a linear relationship with tar levels. Since tryptophan has been shown to produce relatively large quantities of harman and norharman upon pyrolysis, we examined pyrolyzates of various protein-enriched or protein-free fractions from the homogenized leaf curing (HLC) process using the above method. The relative importance and contribution of each of these fractions towards the production of harman and norharman will be discussed..

REVIEW: Dr. Snook discussed the analysis of harmane and norharmane. The internal standard was triphenylene, the last major peak in the gas chromatogram. The two major peaks before the internal standard were identified as harmane and norharmane; a minor peak between these two was identified as methyl norharmane. Since the two compounds were high boiling, the capillary column was programmed from 180 to 250°C. He also identified a number of additional acyl alkaloids in the earlier part of the chromatogram. Compounds identified were formyl, acetyl, hexonyl and octanonyl nornicotine, and formyl and acetyl anatabine. The results for the analysis of harmane and norharmane for four cigarettes with varying tar deliveries are shown below.

	Harmane, ug/cigt.	Norharmane, ug/cigt.	Tar, mg/cigt.
Kentucky Ref. Cigt.	3.7	10.0	34
Nonfilter	2.0	6.5	24
Filter "A"	1.6	4.7	17
Filter "B"	1.5	4.0	8

Dr. Snook showed a slide of the harmane (0.5-2.5 ug/cigt.) and norharmane (2-10 ug/cigt.) contents for a number of commercial cigarettes versus their tar content. The correlation appeared to be quite good. He found no correlation of harmane and norharmane in smoke with the alkaloid content. He also analyzed harmane and norharmane in the smoke of cigarettes made from different types of tobaccos.

	Harmane, ug/cigt.	Norharmane, ug/cigt.
Flue	2.6	10.0
Burley	3.0	12.2
Maryland	2.9	12.4
Turkish	2.7	9.5
2R1	3.6	11.2

Carpenter (1962) reported the addition of labeled tryptophan to tobacco delivered labeled harmane and norharmane in smoke. Dr. Snook pyrolyzed tryptophan and found harmane, norharmane, indole and skatole as major components. Cigarettes were prepared from extract tobacco and deproteinated reconstituted tobacco, and the following results were obtained: in extract tobacco, harmane and norharmane levels (ug/cigt.) were 2.6 and 10.0, respectively; in deproteinated reconstituted tobacco, they were 2.1 and 5.8.

Since the deproteinated reconstituted tobacco still delivered some harmane and norharmane, he stated that the deproteination process was incomplete. Three protein fractions isolated from tobacco were pyrolyzed and analyzed for harmane and norharmane as shown below:

Protein Fractions	Harmane	Norharmane
Green	12.8	58.6
Purified White	16.1	68.9
Purified Chloroplast	13.3	45.5

Dr. Snook showed the values he obtained for harmane and norharmane in cigarette smoke agreed with the values reported by Carpenter. He also verified Carpenter's earlier work on the precursor for harmane and norharmane.

-Review by R. Ikeda